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First DNA-based record of *Arachnocephalus vestitus* Costa, 1855 (Orthoptera: Mogoplistidae) from Georgia

Eka Arsenashvili[1], Natalia Bulbulashvili[2], Lasha-Giorgi Japaridze[3]

1 *Institute of Ecology, Ilia State University, Cholokashvili Ave. 3/5 Tbilisi, 0162, Georgia*

2 *Rustaveli st. 8, 1400, Gori, Georgia*

3 *David Reqtori st. 55, 2200, Telavi, Georgia*

http://zoobank.org/63ECABC8-98F0-4AA5-A126-E59EFED91E1D

Corresponding author: Eka Arsenashvili (eka.arsenashvili.1@iliauni.edu.ge)

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Abstract

The widespread Mediterranean cricket, *Arachnocephalus vestitus* (Orthoptera: Mogoplistidae), is hereby reported as a new record for Georgia. In total, four females have been examined in 2020 and 2021, of which three specimens have been collected, and the information on the fourth one is based on photographic evidence. Cytochrom oxydase I (COI) barcoding further validates our finding.

Key words

Hairy Scale-cricket, CaBOL, Caucasus, Barcoding, Ensifera, New Record

Introduction

The Mogoplistidae includes small and non-flying crickets with their bodies covered by the numerous squamellae, which are most diverse in tropical forests (Ingrisch 2006). The world fauna of Mogoplistidae consists of 31 extant genera. The genus *Arachnocephalus* contains 26 species, distributed on all continents except for North and South America (Cigliano et al. 2022). The only representative of the genus *A. vestitus* (Costa 1855) is known from the Iberian Peninsula and western North Africa; the Mediterranean region from southern France and Corsica, up to Romania, Crimea, the Caucasus and Asia Minor (Heller et al. 1998; Gorochov and Llorente 2001; Cigliano et al. 2022). The Hairy Scale-cricket is found in dry grassland, bushes, and trees (Hochkirch et al. 2016). This species is quite common but difficult to find (Massa 2011), and no information on the population trend of this species is available. *Arachnocephalus vestitus* was first observed in the Caucasus in Azerbaijan (Tarbinsky 1940), but has not been recorded from Georgia so far (Ramme 1951; Shengelia 1956; Mulder and Mulder 2020). In the present contribution, we provide morphological and genetic evidence of the occurrence of this species in eastern Georgia.

Materials and methods

During fieldwork supported by the BMBF-funded project "Caucasus Barcode of Life" (CaBOL – https://ggbc.eu/) in the years 2020 and 2021, three specimens of *Arachnocephalus vestitus* (Fig. 1) were collected by hand in the surroundings of Tbilisi and Gori (both in east Georgia). Specimens were preserved in 96% ethanol and later stored in a freezer under -30°C for further DNA barcoding at Ilia State University, Institute of Ecology. One additional record from village Dzirkoki (Fig. 2) is based on the photographic material posted by Mr Giorgi Cankashvili in the Facebook group "Wildlife in Georgia". This latter specimen has been determined and a georeferenced location was added to the Georgian Biodiversity Database (Tarkhnishvili and Chaladze 2022).

Photos of the preserved specimen were taken using a Canon EOS 90D camera with a Canon EF-S 60 mm f/2.8

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Figure 1. *Arachnocephalus vestitus* from Georgia, Gori, 02.11.2020. The scale bar – 1 mm. (Photo credit: Armen Seropian).

Table 1. Sampling data of the four females of *Arachnocephalus vestitus* from Georgia, providing coordinates and altitude, date of sampling, number of individuals collected (N), sex, and voucher IDs of CABOL and BOLD.

Location	Latitude, Longitude	Altitude (m a.s.l.)	Date	N	Sex	CaBOL-ID	BOLD Process ID
Tbilisi	41.7300°, 44.7048°	710	02.XI.2020	1		1010346	ORTGE001-22
Gori	41.9750°, 44.0994°	664	31.X.2020	1		1010358	ORTGE002-22
Gori	41.9740°, 44.1023°	634	19.IX.2021	1		1016908	–
Dzirkoki	41.7137°, 45.8289°	450	26.VII.2021	1		–	–

Macro USM lens. The digital images were prepared using Zerene Stacker image stacking software and Adobe Photoshop CS6 (Fig. 2). Additional information on studied material is given in Table 1.

DNA processing

Genomic DNA was extracted from tissue samples using the Quick-DNATM Miniprep PlusKit (Zymo Research) (for 25 mg tissue). Partial sequences of cytochrome oxidase subunit I (COI) were amplified by polymerase chain reaction (PCR) using the primer pair LCO1490-JJ and HCO2198-JJ (Astrin and Stüben 2008). Thermal conditions included denaturation at 95 °C for 1 min, followed by the first cycle set (15 cycles): 94 °C for 30 sec, annealing at 55 °C for 1 min (–1 °C per cycle) and extension at 72 °C for 1:30 min. Second cycles set (25 cycles): 94 °C for 35 sec, 45 °C for 1 min, 72 °C for 1:30 min, followed by 1cycle at 72 °C for 3 min and final extension step at 72 °C for 5 min. PCR amplicons were visualized on 1% agarose gels using 1.7 µl of PCR product. The sequencing of the unpurified PCR products in both directions was conducted at the Beijing Genomics Institute (Hong Kong, CN) by using the amplification primers. Sequence analysis was performed using Geneious Prime 2022.1.1 (http://www.geneious.com) and quality sequences were uploaded to BOLD Systems (https://www.boldsystems.org/). Vouchers and extracted DNA samples were deposited in the scientific collections of Ilia State University, Tbilisi, Georgia.

Results and discussion

For the first time, we were able to confirm the occurance of *Arachnocephalus vestitus* Costa, 1855 in Georgia based on morphological analysis and DNA-barcoding. The generally brown, hairy body, with a length of 6 to 9 mm, is covered with small scales that fall off on contact. Head with a projection between the antennae, divided by a longitudinal incision; posterior tibiae saw-shaped but without distinct spines; ovipositor sinuous; shorter than the cerci (Serrano et al. 2015).

Collected specimens of *Arachnocephalus vestitus* were successfully sequenced and 658 bp long sequences were submitted to the BOLD System (Process ID: ORTGE001- 22; ORTGE002-22). The BOLD identification tool has placed our specimens within the group of sequences marked as BIN (barcode index number): AAP5182, which includes specimens of *A. vestitus* from Bulgaria and France (average distance: 0.45% (*p*-dist), maximum within-BIN distance: 0.87% (*p*-dist), and distance to nearest neighbor BIN: 2.41% (*p*-dist)).

Specimens of *Arachnocephalus vestitus* were collected and observed in steppe habitats in Tbilisi, Gori, and Dzirkoki village. Given the sampling sites and cryptic dwelling lifestyle of the hairy scale-cricket, we can assume that the species is not so rare and might be found elsewhere. We assume that it is widely distributed in at least eastern Georgia and has been overlooked in the past, mainly due to the lack of biodiversity research in the country.

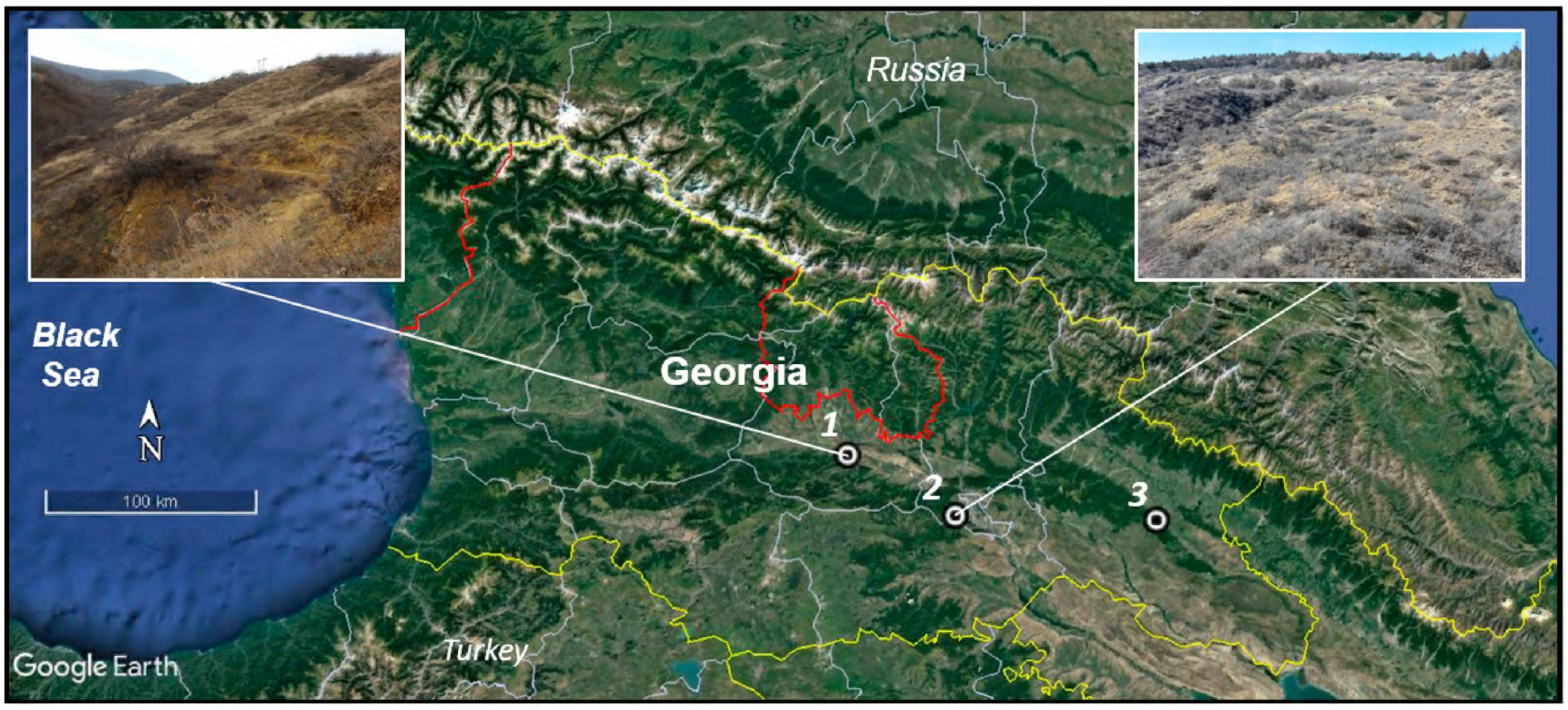

Figure 2. Distribution map of *Arachnocephalus vestitus* in Georgia. 1 - Gori; 2 - Tbilisi; and 3 – Dzirkoki village. Additional data can be found in Table 1.

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